Consider again the bone scaffold product, “Mend-a-bone,” that was described in the first homework set. You successfully convinced the surgeon that he can use this scaffold to transplant marrow cells into a bone defect ONLY if he washes out the glycercol first. This leaves him with a porous, sponge-like scaffold that can be seeded with a marrow cell suspension. The cells then adhere to the scaffold and are stabilized for transplant into a bone defect.

Your surgeon colleague has become convinced to consider the engineering aspects of marrow cell transplantation. He has a patient who was in a motorcycle accident, and she needs multiple bone grafts. He decides he will treat two of the defects with “Mend-a-bone” seeded with the patient’s marrow. One of the defects, in a long bone, is ~4 cm thick. The other defect, in a hand bone, is only ~0.5 cm thick.

His concern is that if he puts too many marrow cells in the scaffold, diffusion of nutrients might limit cell survival. He emails you to ask what cell concentrations are suitable for each of the grafts.

After getting some more information from him, you decide to model the graft as a slab of thickness 2L containing uniformly distributed cells at a concentration of Nc cells/cm³. You suspect that either oxygen or a growth factor will limit cell survival in the graft, and that it is present at the surface of the device at a concentration of C_{AO}. You further presume that the cells are consuming the rate-limiting substrate at a zero-order rate $q_A$ (moles/cell/s). Finally, you assume that all the cells have the same uniform volume, which is $10^{-9}$ cm³/cell.

(a) Draw the coordinate system appropriate for mass transfer in this system. Label the boundary conditions, the dimensions, the direction of transport, and the region where the reaction occurs. 

(20 pts.)

![Coordinate System Diagram]

- **Transport from both sides**
- **Boundary Conditions**
  - $C = C_{AO}$ @ $x = 0$ (constant concentration) (+2pts)
  - $\frac{dC}{dx} = 0$ @ $x = L$ (by symmetry) (+3pts)

+ 3pts one side, +2pts second side

+ 5pts

x = 0  

x = L
(b) Derive the appropriate term for the zero-order volumetric consumption rate of “component A” in the graft as a function of the cell concentration $N_c$ cells/cm$^3$ (5 pts.)

Volumetric Consumption rate, $Q_A$, can be expressed in terms of cell concentration, $N_c$, and cellular consumption rate, $q_A$, by the following term:

$$Q_A = q_A \times N_c \quad (+5\text{pts})$$

(c) What maximum cell concentrations would you recommend for each graft size if oxygen is limiting and is present at the surface at $C_{AO} = 0.1 \times 10^{-6}$ mol/cm$^3$ and $q_A = 2 \times 10^{-17}$ moles/cell/s? You may presume that the diffusion coefficient of oxygen at $37^\circ$C in the scaffold is the same as that in tissue, $2 \times 10^{-5}$ cm$^2$/s. (35 pts.)

There were several equally acceptable ways of solving this problem.

The first way of solving the problem was to use the Theile modulus to calculate the cell concentration $N_c$. This is a planar geometry problem, with homogeneous reaction occurring over the diffusion path length. Therefore, the equation we derived in class, involving the Theile modulus for the planar case, is applicable here. If you will remember from class:

$$\frac{C_A(x)}{C_{AO}} = 1 + \Phi^2 \left( \frac{1}{2} \left( \frac{x}{L} \right)^2 - \frac{x}{L} \right) \quad \text{(using the right equation is worth +10pts)} \quad (1)$$

where

$$\Phi^2 = \frac{Q_A L^2}{C_{AO} D_{AB}}, \quad \text{or, in this particular case,} \quad \Phi^2 = \frac{q_A N_c L^2}{C_{AO} D_{AB}} \quad (2)$$

The next step is to solve for $N_c$, the cell concentration in the graft. What we need to do is find a value of Theile Modulus that will give a desired concentration at the center. The concentration at the center you would like to solve for is up to you, as long as you can explain why you’ve chosen that concentration. Most people decided to solve for a concentration of zero at the center, which if you’ll remember from class, results from a value of 2 for the Theile Modulus. Some decided that “physiological” concentration (one-half of $C_{AO}$) was the way to go, as Dr. Griffith mentioned in class, and this can be given with a value of 1 for Theile Modulus.

If you did not remember any of these two things, it is easy to solve for the value of Theile modulus in both situations. The concentration at the center, $C_{AL}$, can be given by evaluating equation (1) at $x = L$. 
\[
\frac{C_{AL}}{C_{AO}} = 1 + \Phi^2 \left( \frac{1}{2} \left( \frac{L}{L} \right)^2 - \frac{L}{L} \right) = 1 - \frac{\Phi^2}{2}
\]

For \( \frac{C_{AL}}{C_{AO}} = 0 \), \( \Phi^2 = 2 \) (using the correct method is worth +10pts)

For \( \frac{C_{AL}}{C_{AO}} = \frac{1}{2} \), \( \Phi^2 = 1 \)

Now that we have figured out the value of Theile modulus, we can solve for \( N_c \) by rearranging equation (2):

\[
N_c = \frac{\Phi^2 C_{AO} D_{AB}}{q_A L^2}
\]

(3)

so, for \( C_{AL}/C_{AO} = 0 \), and for the hand bone defect (\( L = 0.25 \) cm),

\[
N_c = \frac{2 \left( 0.1 \times 10^{-6} \text{ moles/cm}^3 \right) \left( 2 \times 10^{-5} \text{ cm}^2/\text{sec} \right)}{\left( 2 \times 10^{-17} \text{ mol/cell} \cdot \text{sec} \right) (0.25 \text{ cm})^2} = 3.2 \times 10^6 \text{ cells/cm}^3 \] (for correct answer +10pts)

similarly, for \( C_{AL}/C_{AO} = 0 \), and for the long bone defect (\( L = 2 \) cm)

\[
N_c = \frac{2 \left( 0.1 \times 10^{-6} \text{ moles/cm}^3 \right) \left( 2 \times 10^{-5} \text{ cm}^2/\text{sec} \right)}{\left( 2 \times 10^{-17} \text{ mol/cell} \cdot \text{sec} \right) (2 \text{ cm})^2} = 5.0 \times 10^4 \text{ cells/cm}^3 \] (doing it again +5pts)

Halve these answers to figure out the answers for the \( \Phi^2 = 1 \) case.

A second method to solving this problem would be to remember, or solve for by a shell balance, the concentration profile solved for in class:

\[
C_A(x) = \frac{q_A N_C x^2}{2 D_{AB}} - \frac{q_A N_c L x}{D_{AB}} + C_{AO}
\]

(4)

Then, you can evaluate this equation at \( x = L \), plug in the values of all known quantities, and solve for \( N_c \). Usually, if people did the problem this way, they assumed that the concentration at \( x = L \) was zero.
(d) You have some data that suggest the soluble peptide signaling molecule epidermal growth factor (EGF) is required as a cell survival signal for the bone progenitor cells present in marrow, and that cells will die if the concentration of EGF in their environment drops below 0.1 nM. (i.e., 1 x 10^{-10} mol/L or 1 x 10^{-13} mol/cm^3). While the data on consumption rates for EGF are scarce, you find some papers indicating that cells consume roughly 2 molecules of EGF per second. If the concentration of EGF in the fluid at the surface of the device is ~ 1 nM, and you presume that all cells present in marrow consume EGF at the same rate, should you change the suggestions you made in part c for both graft sizes on the basis of diffusion EGF limitations? You may assume that the diffusion coefficient of EGF in culture medium is 1 x 10^{-5} cm^2/s. (40 pts.)

The easiest way to solve this problem is to calculate the Theile modulus that yields a concentration of 0.1 nM at the center, then to solve for $N_c$, and compare this value of $N_c$ to the value obtained in part (c).

Evaluating equation (1) at $x = L$ gives:

$$\frac{C_{AL}}{C_{AO}} = 1 - \frac{\Phi^2}{2} = \frac{0.1 \text{nM}}{1 \text{nM}} = 0.1$$

(right equation, +10 pts, correct Theile, +10pts)

therefore $\Phi^2 = 1.8$

We need to first solve for the cellular consumption rate, $q_A$, to plug in to the Theile modulus equation, (equation 3). Using Avogadros’s number, we can convert consumption rate from molecules/cell/sec to mole/cell/sec.

$$q_A = \left(\frac{2 \text{ molecules}}{\text{cell} \cdot \text{sec}}\right) \left(\frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ molecules}}\right) = 3.321 \times 10^{-24} \frac{\text{mole}}{\text{cell} \cdot \text{sec}}$$

(+5pts for Avogadro’s)

Now, just solve for $N_c$ in both cases:

for the hand bone defect ($L = 0.25$ cm),

$$N_c = \frac{1.8 \left(1 \times 10^{-12} \frac{\text{mole}}{\text{cm}^3}\right) \left(1 \times 10^{-5} \frac{\text{cm}^2}{\text{sec}}\right)}{3.321 \times 10^{-24} \frac{\text{mol}}{\text{cell} \cdot \text{sec}}} \left(0.25 \text{ cm}\right)^2$$

$$= 8.7 \times 10^7 \text{ cells/cm}^3$$

(for correct answer +5pts)

similarly, for $C_{AL}/C_{AO} = 0$, and for the long bone defect ($L = 2$ cm)

$$N_c = \frac{1.8 \left(1 \times 10^{-12} \frac{\text{mole}}{\text{cm}^3}\right) \left(1 \times 10^{-5} \frac{\text{cm}^2}{\text{sec}}\right)}{3.321 \times 10^{-24} \frac{\text{mol}}{\text{cell} \cdot \text{sec}}} \left(2 \text{ cm}\right)^2$$

$$= 1.3 \times 10^6 \text{ cells/cm}^3$$

(doing it again +5pts)
If you get a higher value for $N_c$ in this part, then you know that the graft can support more cells based on EGF considerations alone than based on $O_2$ considerations alone. Therefore you should seed only as much as can be supported by the oxygen, so you would not change your answer from part (c).

If you get a lower number for $N_c$ than in part (c), then EGF is the limiting factor. Therefore, you should change your suggestion, and seed only as much as can be supported by the amount of EGF available.

Since we found both values to be higher for both thicknesses, we would not change our suggestions from part (c). (reasoning +5pts)